Single cell analysis facilitates staging of blimp1-dependent primordial germ cells derived from mouse embryonic stem cells.

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Public Summary:
The cell intrinsic programming that regulates mammalian primordial germ cell (PGC) development in the pre-gonadal stage is challenging to investigate. To overcome this we created a transgene-free method for generating PGCs in vitro (iPGCs) from mouse embryonic stem cells (ESCs). Using labeling for SSEA1 and cKit, two cell surface molecules used previously to isolate presumptive iPGCs, we show that not all SSEA1+/cKit+ double positive cells exhibit a PGC identity. Instead, we determined that selecting for cKit(bright) cells within the SSEA1+ fraction significantly enriches for the putative iPGC population. Single cell analysis comparing SSEA1+/cKit(bright) iPGCs to ESCs and embryonic PGCs demonstrates that 97% of single iPGCs co-express PGC signature genes Blimp1, Stella, Dnd1, Prdm14 and Dazl at similar levels to e9.5-10.5 PGCs, whereas 90% of single mouse ESC do not co-express PGC signature genes. For the 10% of ESCs that co-express PGC signature genes, the levels are significantly lower than iPGCs. Microarray analysis shows that iPGCs are transcriptionally distinct from ESCs and repress gene ontology groups associated with mesoderm and heart development. At the level of chromatin, iPGCs contain 5-methyl cytosine bases in their DNA at imprinted and non-imprinted loci, and are enriched in histone H3 lysine 27 trimethylation, yet do not have detectable levels of Mvh protein, consistent with a Blimp1-positive pre-gonadal PGC identity. In order to determine whether iPGC formation is dependent upon Blimp1, we generated Blimp1 null ESCs and found that loss of Blimp1 significantly depletes SSEA1/cKit(bright) iPGCs. Taken together, the generation of Blimp1-positive iPGCs from ESCs constitutes a robust model for examining cell-intrinsic regulation of PGCs during the Blimp1-positive stage of development.

Scientific Abstract:
The cell intrinsic programming that regulates mammalian primordial germ cell (PGC) development in the pre-gonadal stage is challenging to investigate. To overcome this we created a transgene-free method for generating PGCs in vitro (iPGCs) from mouse embryonic stem cells (ESCs). Using labeling for SSEA1 and cKit, two cell surface molecules used previously to isolate presumptive iPGCs, we show that not all SSEA1+/cKit+ double positive cells exhibit a PGC identity. Instead, we determined that selecting for cKit(bright) cells within the SSEA1+ fraction significantly enriches for the putative iPGC population. Single cell analysis comparing SSEA1+/cKit(bright) iPGCs to ESCs and embryonic PGCs demonstrates that 97% of single iPGCs co-express PGC signature genes Blimp1, Stella, Dnd1, Prdm14 and Dazl at similar levels to e9.5-10.5 PGCs, whereas 90% of single mouse ESC do not co-express PGC signature genes. For the 10% of ESCs that co-express PGC signature genes, the levels are significantly lower than iPGCs. Microarray analysis shows that iPGCs are transcriptionally distinct from ESCs and repress gene ontology groups associated with mesoderm and heart development. At the level of chromatin, iPGCs contain 5-methyl cytosine bases in their DNA at imprinted and non-imprinted loci, and are enriched in histone H3 lysine 27 trimethylation, yet do not have detectable levels of Mvh protein, consistent with a Blimp1-positive pre-gonadal PGC identity. In order to determine whether iPGC formation is dependent upon Blimp1, we generated Blimp1 null ESCs and found that loss of Blimp1 significantly depletes SSEA1/cKit(bright) iPGCs. Taken together, the generation of Blimp1-positive iPGCs from ESCs constitutes a robust model for examining cell-intrinsic regulation of PGCs during the Blimp1-positive stage of development.